
**Water quality — Determination of
glyphosate and AMPA — Method using
high performance liquid chromatography
(HPLC) and fluorometric detection**

*Qualité de l'eau — Dosage du glyphosate et de l'AMPA — Méthode par
chromatographie liquide à haute performance (CLHP) et détection
fluorimétrique*



Reference number
ISO 21458:2008(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 21458 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Water quality — Determination of glyphosate and AMPA — Method using high performance liquid chromatography (HPLC) and fluorometric detection

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions. Some of the solvents used in the procedure are toxic and dangerous. Exercise caution when handling them.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

When this method is applied to its analysis, surface water shall be tested for additional and for multiplicative interferences.

1 Scope

This International Standard specifies a method for the determination of glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), in drinking water, ground water and surface water. The lowest limit of determination is about 0,05 µg/l. This method may be applicable to other types of waters provided the method is validated for each case.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 6058, *Water quality — Determination of calcium content — EDTA titrimetric method*

ISO 6059, *Water quality — Determination of the sum of calcium and magnesium — EDTA titrimetric method*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

3 Principle

This method is based on derivatization of glyphosate and AMPA (see Table 1) by using 9-fluorenylmethyl chloroformate (FMOCCl) in basic conditions followed by analysis using liquid chromatography on a polar phase linked to a fluorescence detector.

The quantification of the compounds is performed by using external calibration or by internal standard calibration or by applying the standard addition method. The compounds are identified by comparing the retention times or by using the standard addition method.

Table 1 — Compounds determined by this method

Name	Molecular formula	Molar mass g/mol	CAS No.
Glyphosate <i>N</i> -(phosphonomethyl)glycine	C ₃ H ₈ NO ₅ P	169,1	1071-83-6
AMPA Aminomethylphosphonic acid	CH ₆ NO ₃ P	111,0	1066-51-9

4 Interferences

Substances that produce a response at the selected wavelengths and for which the retention times are identical to those of the compounds to be analysed may cause overlapping peaks during the determination. It is necessary to take this into account especially when testing water samples other than ground water or drinking water samples.

The presence of divalent cations, such as calcium, copper, iron, and zinc, may lead in some cases to an underestimation of glyphosate and AMPA due to complex formation (see Reference [9]), in which case pretreatment is required (see 8.1).

The presence of free chlorine used in treated waters may cause losses of glyphosate by oxidation, in which case sodium thiosulfate is used to mitigate its effects (see Clause 7).

5 Reagents

Use only reagents of recognized analytical or HPLC grade, if available, unless otherwise specified. Reagents shall not contain any substance that interferes with the compounds to be analysed.

5.1 Water, complying with grade 1 as defined in ISO 3696, or better.

5.2 Sodium thiosulfate, Na₂S₂O₃, CAS No.: 7772-98-7.

5.3 Acetonitrile, CH₃CN, HPLC grade, CAS No.: 75-05-8.

5.4 Diethyl ether, C₄H₁₀O, CAS No.: 60-29-7.

5.5 Reference substances (see Table 1).

5.5.1 Glyphosate, purity > 97 % mass fraction, CAS No.: 1071-83-6.

5.5.2 AMPA, purity > 97 % mass fraction, CAS No.: 1066-51-9.

5.5.3 Internal standard, purity > 97 % mass fraction (see Annex A for typical compounds).

Commercially available solutions may be used, e.g. 10 µg/ml in water.

5.5.4 Stock solution of glyphosate and AMPA, 1,0 g/l.

Using glyphosate (5.5.1) and AMPA (5.5.2), prepare a stock solution containing 1,0 g/l of each compound in water (5.1), e.g. dissolve 20 mg of each compound in water (5.1) in a 20 ml one-mark volumetric flask (6.8).

This solution may be stored at 4 °C ± 3 °C for about 1 year.

5.5.5 Intermediate concentration solution of glyphosate and AMPA, 30 µg/l.

Proceed with subsequent dilutions of stock solution of glyphosate and AMPA (5.5.4) in order to obtain a mass concentration of 30 µg/l.

NOTE The solution can be obtained by adding 200 µl of stock solution (5.5.4) to 20 ml of water (5.1) in a one-mark volumetric flask (6.8); then adding 60 µl of this solution to 20 ml of water (5.1) in another one-mark volumetric flask (6.8).

This solution may be stored at 4 °C ± 3 °C for about 1 week.

5.5.6 Stock solution of internal standard, 30 µg/l.

Dissolve an internal standard (5.5.3) in water (5.1) and dilute to obtain a mass concentration of 30 µg/l in a one-mark volumetric flask (6.8).

This solution may be stored at 4 °C ± 3 °C for about 1 week.

5.6 Potassium hydroxide solution, $c(\text{KOH}) = 3 \text{ mol/l}$, CAS No.: 1310-58-3,.

Dissolve 16,8 g of potassium hydroxide in 70 ml to 80 ml of water (5.1) in a 100 ml one-mark volumetric flask (6.8) and make up to the mark with water.

This solution may be stored at 4 °C ± 3 °C for about 3 months.

5.7 Hydrochloric acid, $c(\text{HCl}) = 4 \text{ mol/l}$, CAS No.: 7647-01-0.

To 340 ml of water (5.1), slowly add 160 ml of concentrated HCl (37 % mass fraction) under magnetic stirrer agitation in a 500 ml one-mark volumetric flask (6.8). This solution may be stored at 4 °C ± 3 °C for about 3 months.

5.8 Potassium dihydrogenphosphate solution, $c(\text{KH}_2\text{PO}_4) = 0,05 \text{ mol/l}$, pH 5,4, CAS No.: 7778-77-0.

Dissolve 6,8 g ± 0,05 g of potassium dihydrogenphosphate in 1 000 ml of water. Adjust to pH = 5,4 ± 0,05 with potassium hydroxide solution (5.6).

This solution may be stored for about 1 week.

5.9 Oxalic acid dihydrate solution, $\rho(\text{C}_2\text{H}_2\text{O}_4 \cdot 2 \text{H}_2\text{O}) = 100 \text{ g/l}$, CAS No.: 6153-56-6.

Dissolve 50 g ± 0,05 g of oxalic acid dihydrate in 500 ml of water.

This solution may be stored at 4 °C ± 3 °C for about 2 months.

5.10 Mobile phase.

Mix 300 ml acetonitrile (5.3) and 700 ml of potassium dihydrogenphosphate buffer (5.8).

This solution can be stored for about 1 week.

5.11 Sodium tetraborate decahydrate solution, $c(\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}) = 0,05 \text{ mol/l}$, CAS No.: 1303-96-4.

Dissolve 19 g ± 0,1 g of sodium tetraborate decahydrate in 1 000 ml of water.

This solution may be stored at 4 °C ± 3 °C for about 1 month.

5.12 9-Fluorenylmethyl chloroformate solution, $\rho(\text{C}_{15}\text{H}_{11}\text{ClO}_2) = 1 \text{ mg/ml}$, CAS No.: 28920-43-6.

Dissolve 50 mg of 9-fluorenylmethyl chloroformate (FMOCCL; 97 % mass fraction) in 50 ml of acetonitrile (5.3).

This solution may be stored at 4 °C ± 3 °C for about 2 weeks.

5.13 Phosphoric acid, 85 % (acidimetric), CAS No.: 7664-38-2.

6 Apparatus

Equipment or components that may come into contact with the sample shall be free from any residue that could cause unacceptable interference in the blanks.

The use of glassware may cause losses of glyphosate by adsorption. Consequently, the use of disposable materials is recommended wherever possible (see 6.1 and 6.2). For the same reason, check that any filters used for samples prior to HPLC analysis do not lead to losses of substances.

6.1 Disposable plastic test tubes with screw caps, of capacity about 20 ml, for the derivatization.

6.2 Polyolefin bottles, of capacity about 50 ml, for sampling.

6.3 Syringe, for the standard solution of glyphosate and AMPA at the mass concentration 30 µg/l (5.5.5) and internal standard at a mass concentration of 30 µg/l (5.5.6).

6.4 Analytical column: polar phase column: e.g. silica-bonded NH₂ type chromatographic column, particle size 3 µm to 5 µm, inner diameter 2 mm to 4,6 mm, length 250 mm. A suitable guard-column is recommended.

Other types of columns may be used, e.g. reverse phase C₁₈ column, material with particle size 3 µm to 5 µm inner diameter 2 mm to 4,6 mm, length 250 mm. See elution conditions in Figure C.2 when using C₁₈ column.

6.5 Vials, standard 2 ml vials with polytetrafluoroethylene caps.

CAUTION — Do not clean vials with any detergent or corrosive agents before use.

6.6 High performance liquid chromatograph (HPLC), including:

6.6.1 System, allowing the elution of mobile phase (5.10) with either manual or automatic injection designed for analytical work.

6.6.2 Degassing system.

6.6.3 HPLC column oven with temperature control unit, 35 °C.

6.6.4 Fluorescence detector, $\lambda_{\text{exc}} = 260 \text{ nm}$; $\lambda_{\text{em}} = 310 \text{ nm}$.

6.7 pH meter.

6.8 One-mark volumetric flasks, ISO 1042 [10] class A, of capacities 20 ml, 100 ml, 500 ml.

CAUTION — Do not clean one-mark volumetric flasks with any detergent or corrosive agents before use.

7 Sampling

Fill the polyolefin bottles (6.2) to the top with the water to be analysed.

For samples containing free chlorine, add about 2 mg of sodium thiosulfate (5.2) or any other chlorine reducing agent per 100 ml of sample.

The water samples to be analysed may be stored in the dark at 4 °C ± 2 °C for 1 week or frozen in polyolefin bottles (6.2) for 1 month. The freezer temperature should be at least -20 °C.

8 Procedure

8.1 Pretreatment

Determine the water hardness as specified in ISO 6058 and/or ISO 6059. If the measure of hardness, $c(\text{CaCO}_3)$, exceeds 3 mmol/l, a sample pretreatment is recommended, in order to release any test substance complexed with divalent cations (Reference [9]). Two preferred pretreatment procedures are proposed (8.1.1 and 8.1.2), the choice of which is left to the user. A third option for pretreatment can be suitable in some cases (8.1.3).

8.1.1 First option for pretreatment

Adjust 3 ml of sample to pH 1 with about 200 μl of hydrochloric acid (5.7). Shake the mixture vigorously and wait for 1 min. Adjust the sample to between pH 6 and pH 7 with potassium hydroxide solution (5.6). Then proceed as specified in 8.2.

8.1.2 Second option for pretreatment

Add 300 μl of oxalic acid dihydrate solution (5.9) to 30 ml of sample. Check that the pH is between 2 to 3. Shake the mixture vigorously and wait for 1 h. Neutralize the sample with potassium hydroxide solution (5.6). Then proceed as specified in 8.2.

8.1.3 Third option for pretreatment

Add 0,625 ml of sodium tetraborate decahydrate buffer (5.11) and 50 μl of EDTA (1 g/l, pH = 9 adjusted by the addition of potassium hydroxide) to 4 ml sample. Shake the mixture. Store it for 24 h at $4\text{ }^\circ\text{C} \pm 3\text{ }^\circ\text{C}$. Without shaking, take 3 ml of the clear sample. Then proceed as described in 8.2.

8.2 Derivatization

After thawing the sample, if frozen (see Clause 7), mix the solution thoroughly and withdraw an aliquot of 3 ml. Transfer into a disposable plastic test tube with screw cap (6.1). Add 50 μl of the internal standard (5.5.6) to achieve a mass concentration of about 0,5 $\mu\text{g/l}$.

Add 0,5 ml of sodium tetraborate decahydrate buffer (5.11) and let it stand for about 15 min.

Add about 3 ml of diethyl ether (5.4), mix vigorously for 2 min and allow to settle for 15 min.

Take 1,5 ml from the aqueous phase and add 0,25 ml of acetonitrile (5.3), then add 0,25 ml of FMOCCI (5.12).

Allow the mixture to react at ambient temperature for 60 min. Stop the derivatization by adding 40 μl phosphoric acid (5.13) and mix.

NOTE 1 A reaction time of 30 min is sufficient to reach a yield of 100 %, however, the last 30 min allow for a better elimination of excess reagent. An excess of reagent can lower the life of the analytical column and also causes interferences.

NOTE 2 For some samples, using a slow derivatization results in fewer interfering peaks in the chromatogram. Leave out the extra acetonitrile as an accelerant. Allow the mixture to react at ambient temperature for 18 h. Stop the derivatization by adding 40 μl phosphoric acid (5.13) and mix.

Add 2 ml of diethyl ether (5.4) and mix for 2 min, allow the mixture to stand for 1 h and transfer an aliquot of the aqueous phase to a vial (6.5) for analysis. Analyse the aqueous extract or store it at $4\text{ }^\circ\text{C} \pm 3\text{ }^\circ\text{C}$ for up to 2 weeks (4 weeks in a freezer).

If the standard addition approach is used to quantify compounds, prepare three different plastic test tubes with screw caps as described in Table 2. Allow the solutions to stand for 15 min then proceed with derivatization.

Table 2 — Preparation of spiked samples, in 3 different plastic test tubes with screw caps (6.1)

	Mass concentration of fortification (0 µg/l; 0,5 µg/l and 1 µg/l)		
	0,5 ml	0,5 ml	0,5 ml
Sodium tetraborate buffer (5.11)	0,5 ml	0,5 ml	0,5 ml
Samples	3 ml	3 ml	3 ml
Intermediate concentration solution of glyphosate and AMPA, µg/l (5.5.5)	0 µl	50 µl	100 µl

8.3 High performance liquid chromatography (HPLC)

8.3.1 General requirements

Use equipment in accordance with the instructions provided by the manufacturer. Make sure that the background noise of the detector is low and the baseline drift is not significant. Due to the use of buffer-containing mobile phase, regular rinsing of the analytical column and the chromatographic system according to the manufacturer's instructions is recommended.

8.3.2 Chromatographic conditions

Separate the glyphosate and AMPA derivative by HPLC using a reversed phase column and appropriate working conditions (for examples see 5.10, 6.4 and Annex C). Adjust the eluent flow rate and the injection volume according to the column dimensions (inner diameter, particle size) to obtain the maximum peak shape and resolution. Optimum wavelengths for acquisition are 260 nm for excitation and 310 nm for emission.

8.4 Blank monitoring

For the quality control of the analytical procedure, determine the contribution of the reagents and the material by analysing an equivalent amount of water (5.1) in the same way as the sample. In the presence of interfering peaks in the blank solution (generally more than 10 % of the lowest measured value), systematically investigate the sources of contamination in order to be able to eliminate them.

8.5 Confirmation and identification of substances

Identify a target compound in the chromatogram by comparing its retention time with the retention time of the reference substance. For proper identification, the retention time should be within ± 1 % (± 10 s) from the retention time of the latest run of calibration solution. When internal standards are used, the retention of the internal standards can be used to confirm the absolute retention of the analyte.

In the absence of a peak in the normal retention time window and in the presence of a normal chromatogram in all other aspects, the conclusion can be drawn that the substance is absent.

If peak broadening occurs, the analytical conditions can be modified in order to effect separation of the interference, or a C₁₈ column could then be used instead of the NH₂ column.

NOTE The decrease in retention time of glyphosate and AMPA with each injection requires the frequent injection of standard solutions, for instance after every six samples.

9 Calibration

9.1 Calibration with external standard covering the overall procedure

Derivatization and analysis are performed *in situ*. It is not necessary to determine the derivatization yields as long as the standards and the samples are analysed under the same conditions.

Prior to each analytical series, establish the calibration function from at least 5 points as specified in ISO 8466-1 in the working range (0,05 µg/l to 2,0 µg/l) using intermediate standard solution (5.5.5). It is preferable to use reference water (with a known and constant hardness, as close to samples as possible) as calibration matrix.

Establish a calibration function for each compound.

Plot the information values, y_{ie} (peak area, or integration units, depending on the situation), on the ordinate for each substance and the corresponding mass concentration, ρ_{ie} , on the abscissa.

Determine the linear calibration function, Equation (1), using the co-ordinates (ρ_{ie} , y_{ie}) from the measurement series. The measured value of substance i , y_{ie} , the unit depending on the evaluation, e.g. peak area, obtained from the calibration, is given by

$$y_{ie} = a_i \rho_{ie} + b_i \quad (1)$$

where

a_i is the slope of the calibration curve for substance i (corresponding to the coefficient of the specific response of the substance), e.g. peak area, in litres per microgram;

b_i is the intercept on the ordinate of the calibration curve for substance i ;

ρ_{ie} is the mass concentration, in micrograms per litre, of substance i .

9.2 Calibration with internal standard covering the overall procedure

The use of an internal standard to determine the concentrations of glyphosate and AMPA minimizes possible errors made during injection or by sample losses during sample pretreatment steps, differences in the final aqueous extract volumes and matrix effects. This calculation is usually available as an option in the quantification programs of most manufacturers' data analysis software.

Establish a calibration function for each compound before each analytical series.

Plot the measured values, $y_{ie}/y_{int,ie}$, in peak area or integration units, depending on the situation, on the ordinate for each substance and the corresponding mass concentration, $\rho_{ie}/\rho_{int,ie}$ on the abscissa.

Determine the linear calibration function, Equation (2), using the co-ordinates ($\rho_{ie}/\rho_{int,ie}$, $y_{ie}/y_{int,ie}$) from the measurement series. The ratio of the measured responses, e.g. as peak areas, from the calibration to the internal standard for substance i , $y_{ie}/y_{int,ie}$ is given by

$$\frac{y_{ie}}{y_{int,ie}} = a_i \frac{\rho_{ie}}{\rho_{int,ie}} + b_i \quad (2)$$

where

a_i is the slope of the calibration curve for substance i ;

b_i is the intercept on the ordinate of the calibration curve for substance i ;

ρ_{ie} is the mass concentration, in micrograms per litre, of substance i ;

$\rho_{int,ie}$ is the mass concentration, in micrograms per litre, of internal standard for the substance i .

10 Evaluation

10.1 Calculation of single results by calibration with external standard covering the overall procedure

Calculate the mass concentration, ρ_{ig} , in micrograms per litre, of substance i in the water sample from Equation (3):

$$\rho_{ig} = \frac{(y_{ig} - b_i)}{a_i} \quad (3)$$

where

a_i is the slope of the calibration curve for substance i (corresponding to the coefficient of the specific response of the substance), e.g. peak area, in litres per microgram;

b_i is the intercept on the ordinate of the calibration curve for substance i ;

y_{ig} is the response for substance i , e.g. measured area, in the sample.

10.2 Calculation of single results by calibration with internal standards covering the overall procedure

Calculate the mass concentration, ρ_{ig} , in micrograms per litre, of substance i in the water sample from Equation (4).

$$\rho_{ig} = \frac{[(y_{ig} / y_{int,ig}) - b_i]}{a_i} \rho_{int,ig} \quad (4)$$

a_i is the slope of the calibration curve for substance i ;

b_i is the intercept on the ordinate of the calibration curve for substance i ;

y_{ig} is the response for substance i , e.g. measured area, in the sample;

$y_{int,ig}$ is the measured area of internal standard for substance i in the sample;

$\rho_{int,ig}$ is the mass concentration, in micrograms per litre, of internal standard for substance i in the water sample.

10.3 Calculation of single results by calibration with standard addition covering the overall procedure

Calculate the mass concentration, ρ_{ig} , of glyphosate and AMPA. In the standard addition procedure, the mass concentration of substances is given by the abscissa intercept value.

The standard addition method provides the analyst with a powerful tool for increasing the accuracy of a fluorometric detection method. It can be applied to hard water [$c(\text{CaCO}_3) > 3 \text{ mmol/l}$]. The general requirement of standard addition can be found in the literature on atomic absorption.

Plot the response of substance i for the solutions prepared according to Table 2 against the mass concentration, in micrograms of substance per litre of solution. Extrapolate the straight line to intersect the abscissa. At the point of intersection, read the concentration of substance i in the test solution.

11 Expression of results

Express the concentration of each compound in micrograms per litre with at most two significant figures.

EXAMPLES	Glyphosate	0,15 µg/l
	AMPA	1,2 µg/l

12 Test report

The test report shall contain at least the following information:

- a) a reference to this International Standard (ISO 21458);
- b) identification of the location from which the water samples were taken;
- c) the time elapsed between sampling and derivatization;
- d) the results, as specified in Clause 11;
- e) any deviation in relation to this procedure and any special circumstances that could have affected the results;
- f) any extra treatment, for example, filtration.

Annex A
(informative)

Internal standard generally used

A.1 L-Cysteic acid monohydrate, $C_3H_7NO_5S \cdot H_2O$, CAS No.: 23537-25-9.

WARNING — This internal standard is only useful to monitor retention time shifts and for checking derivatization takes place.

A.2 O-Phospho-L-serine, $C_3H_8NO_6P$, CAS No.: 407-41-0.

A.3 2-Aminoethylphosphonic acid, $C_2H_8NO_3P$, CAS No.: 2041-14-7.

Annex B (informative)

Precision data

An international interlaboratories test performed on real water samples during the 2nd quarter of 2007 (Reference [8]), provided the results shown in Table B.1.

Table B.1 — Precision data

Sample	Substance	No. labs, n_l	No. measurements, n_m	Percentage of outliers, p_o	Reference concentration, ρ_{ref}	Total mean concentration, $\bar{\rho}$	Coefficient of variation of repeatability, $CV(r)$	Repeatability standard deviation, s_r	Coefficient of variation of reproducibility, $CV(R)$	Reproducibility standard deviation, s_R
				%	$\mu\text{g/l}$	$\mu\text{g/l}$	%	$\mu\text{g/l}$	%	$\mu\text{g/l}$
1. Drinking water, low	Glyphosate	25	83	21	0,100	0,103	5,1	0,005	28,6	0,029
	AMPA	25	85	17	0,116	0,118	5,0	0,006	30,0	0,035
2. Drinking water, high	Glyphosate	25	85	0	0,816	0,803	4,1	0,033	29,8	0,239
	AMPA	25	87	13	0,958	0,949	3,8	0,036	21,2	0,201
3. Ground water, low	Glyphosate	25	83	13	0,204	0,194	6,1	0,012	31,9	0,062
	AMPA	25	85	4	0,240	0,209	7,4	0,015	29,2	0,061
4. Ground water, high	Glyphosate	25	85	17	1,632	1,574	4,5	0,071	33,7	0,531
	AMPA	25	87	21	1,917	1,932	3,7	0,071	17,0	0,329
5. Surface water, low	Glyphosate	25	83	21	0,510	0,558	3,6	0,020	29,9	0,167
	AMPA	25	87	8	0,599	0,790	5,6	0,044	17,1	0,135
6. Surface water, high	Glyphosate	25	85	8	5,100	4,720	5,6	0,264	37,9	1,790
	AMPA	25	83	8	5,990	6,180	3,8	0,235	24,3	1,504

The samples used for this exercise were characterized for cations, especially those impairing hardness (see Table B.2).

Table B.2 — Characterization of test samples

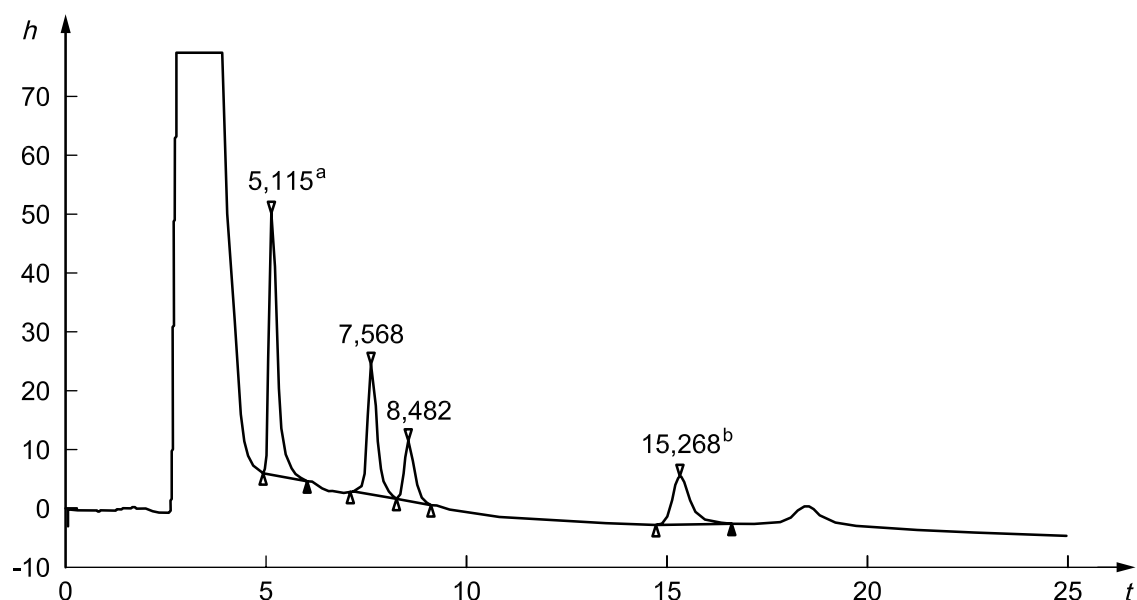
Matrices	[Na ⁺] mg/l	[K ⁺] mg/l	[Mg ²⁺] mg/l	[Ca ²⁺] mg/l
Drinking water	7,1 ± 0,9	0,53 ± 0,05	41 ± 6	141 ± 21
Ground water	15 ± 2	1,1 ± 0,1	44 ± 6	166 ± 25
Surface water	12 ± 1	2,4 ± 0,2	11 ± 2	195 ± 30

Annex C (informative)

Chromatograms

NOTE NH_2 -type columns are fragile and have a short lifetime. The retention time of glyphosate and AMPA decreases with each injection. These columns appear to last for approx. 150 samples.

Column	Silica bonded:	NH_2
	Particle size:	3 μm
	Inner diameter:	4,6 mm
	Length:	250 mm
	Temperature:	35 °C
	Injection volume:	50 μl
Type of sample	Surface water:	2,5 pg injected
Retention times	Glyphosate; AMPA	15,268 min; 5,115 min
Fluorescence detector	260 nm for excitation and 310 nm for emission	PMT voltage (medium)
Mobile phase	acetonitrile and potassium dihydrogenphosphate buffer 0,05 mol/l, pH 5,4:	30 % acetonitrile 70 % potassium dihydrogenphosphate
	Flow rate:	1 ml/min

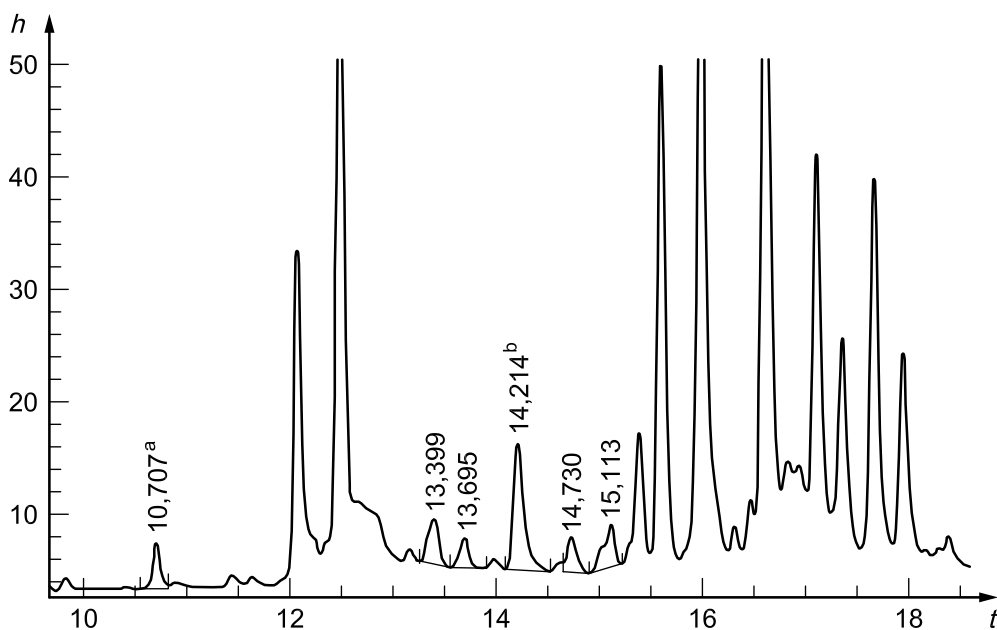


Key

- h height
- t time, min
- a AMPA.
- b Glyphosate.

Figure C.1 — Chromatogram of surface water (glyphosate and AMPA around 2,5 pg injected) with NH_2 separation

Column	Silica bonded:	C ₁₈
	Particle size:	5 µm
	Inner diameter:	3 mm
	Length:	250 mm
	Temperature:	27 °C
	Injection volume:	25 µl
Type of sample	Surface water:	0,25 ng injected
Retention times	Glyphosate; AMPA	10,707 min; 14,214 min
Fluorescence detector	260 nm for excitation and 310 nm for emission	
Mobile phase	A: Potassium dihydrogenphosphate buffer (0,002 mol/l potassium dihydrogenphosphate adjusted to pH 7,0 with potassium hydroxide)	
	B: Acetonitrile	
	0 min to 25 min:	93 % to 43 % A
	25 min to 27 min:	43 % to 5 % A
	27 min to 35 min:	5 % A
	35 min to 37 min:	5 % to 93 % A
	Flow rate:	1 ml/min

**Key**

- h* height
t time
a Glyphosate.
b AMPA.

Figure C.2 — Chromatogram of surface water (glyphosate and AMPA injected about 0,25 ng) with C₁₈ separation

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